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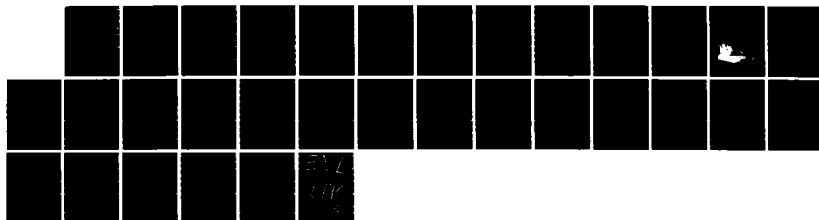
SEMICONDUCTING CHEMICAL MICROSENSORS USING BIOANALOGOUS 1/1
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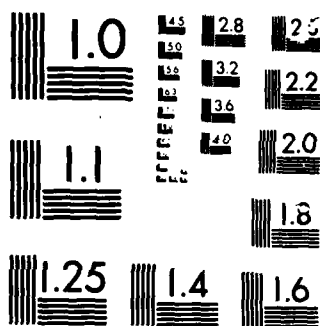
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I. GENERAL OBJECTIVES

The objectives of our research on the d.c. conductivity of proteins was to investigate and demonstrate that appropriate electrical properties of macromolecular substates of biological origin can be exploited for analytical purposes using macroscopic techniques. More concretely, we wanted to show that the specificity and selectivity of specific antibodies in molecular (target) recognition and binding, combined with monitoring binding-induced changes in their d.c. electric conductivity may represent such properties. If so, at least these biomolecular systems can be considered as potential sensory elements in designing chemically highly selective and very sensitive chemical microsensors. Such chemically sensitive gate elements of appropriate biomolecules in F(ield) E(ffect) T(ransistor) - type devices could then be used for the real-time evaluation of vanishingly small quantities of diverse molecular species present in the vapor (gas) or liquid form.

II. TIME PERIOD

Our previous reports described the progress achieved during the various phases of this work, until June 30, 1985. The first report covering the period September 1, 1982-June 10, 1983 dealt with the various aspects of sample preparation using bovine serum albumin and derivatized bovine serum albumin powder samples. These

Contd. 1985

experiments provided background information required to define an appropriate reference system. The work during October and November, 1983 was supported via the Jet Propulsion Laboratory (Pasadena, CA) and a Final Report was submitted to them. These results were also incorporated in the Progress Report prepared for the period June 10, 1983-June 30, 1984. These results formed the basis of sample miniaturization which turned out to be a prerequisite of the successful work with monoclonal antibodies. The various aspects and problems of miniaturization as well as the experimental reasons for choosing thin protein film samples cast over a hairline were presented in the Progress Report for July 01 - December 31, 1984 and, in part, in The Progress Report covering the first half (January 01-June 30) of year 1985. In this latter report preliminary data for monoclonal antibodies were also given and some special problems which we encountered while using immunoproteins were noted.

It became clear during February 1986 that a further no-cost extension of the program will not be made beyond February 28, 1986. Thus, the Progress Report which was due in January was not prepared and submitted separately. It was decided that it would be incorporated into the Final Report; they are combined and presented here. The present Final Report therefore covers the whole period between July 01, 1985 and February 28, 1986 and includes the final conclusions of our efforts.

III. MEASUREMENTS ON MINIATURE (ULTRATHIN) PROTEIN SAMPLES

In our previous measurements /1/ on compacted powder samples of protein it has been found that the covalent binding of a single molecule of a low-molecular-weight organic compound (in our case 2,4-dinitro phenol, 2,4-DNP; m.w. 287) to a medium sized protein molecule (bovine serum albumin, BSA; m.w. 68,000) can be detected using relatively simple electric measurements. Notably, the d.c. electric conductivity of the chemically modified protein (derivatized BSA) and that of the unmodified BSA differ significantly in the pressure range of 0-650 MPa, if the samples are equilibrated with controlled humidity atmosphere of less than 25% relative moisture content. In measurements on powdered protein samples, however, relatively large quantities (up to 200 mg) are needed of the protein and, the theoretically expected high specific surface (surface-to-volume ratio) is "lost" due to compaction and sample geometry (sandwich) required by this technique. These circumstances make the otherwise attractive and promising possibility impractical if immunoproteins are intended to be used. Even, the least expensive, specific monoclonal antibody which is purchasable in sufficient purity and large enough quantities, the Monoclonal Mouse Anti-DNP IgE Antibody (from Miles Scientific, Division of Miles Laboratories, Inc.) is available at a price of \$210/mg. Therefore various techniques for sample miniaturation have been tested /2,3/.

Our results on the conductivity properties of hydrated protein powders /1/ revealed that the overall conductivity of

unmodified protein samples in powder form can be attributed to two well distinguishable phenomenological processes: an interfacial one which occurs near the macroscopic electrode - protein pellet interface and, a bulk one which is due to the charge carrier motion within the macroscopic bulk of the sample. These interfacial and bulk properties are different from those of either the individual protein grains or the protein molecules themselves. Both of these conductivity components increase by several orders of magnitude upon hydration. However, while the interfacial conductivity was found practically independent of, the bulk one showed a rather rapid, exponential decrease with, external pressure applied in an one-sided way, up to 500 MPa. These observations have at least three immediate implications pertinent to our original research objectives:

- (1) Apparently both the interfacial and the bulk conductivity respond very sensitively to adsorption of polar molecules (water, alcohols, etc.) and therefore either of these features could be used to monitor the effects of binding of small molecules on the electric charge transport in proteins.
- (2) Performing measurements on thin-layer protein samples whose conductivity is (predominantly) interfacial in nature, the uncertainties connected with the influence of, and inherent to, the granular structure of powder samples could be greatly reduced because the contribution of pressure-dependent grain-boundary, compaction, etc.

phenomena would be minimum.

- (3) Thin protein layers on insulating substrate equipped with closely-lying surface electrodes (with electrode gap of the order of 20-100 μ) may offer a feasible way towards sample size reduction.

Keeping these in mind, conductivity measurements were carried out on various thin film protein samples cast over two silver electrodes on quartz substrate, separated by a hairline typically of about 40 μ in width. BSA, BSA-2,4-DNP, Monoclonal Mouse Anti-DNP IgE (MM anti-DNP IgE) antibody and its respective immunocomplexes with 2,4-DNP-lysine, and picric acid, respectively, were used at hydrations acquired at ambient atmospheres with lower than 25% relative humidity. Measurements on thin films of BSA and BSA derivatives confirmed our earlier findings with pelleted samples: 2,4-DNP-loaded BSA exhibited significantly lower conductivity even at protein (BSA) - 2,4-DNP stoichiometry as low as 1:2. These experiments which formed the basis of the work on thin layers of MM anti-DNP IgE antibody, were performed as follows:

EXPERIMENTAL ASPECTS

BSA (Fraction V; Sigma Chemical Co.) was derivatized in 0.15 M aqueous K_2CO_3 solution, incubating with various calculated amounts of recrystallized 2,4-dinitrobenzenesulfonic acid (2,4-DNB-SA) in the dark for 24 hours at room temperature and under continuous stirring /4,5/. To remove ionic and other

contaminations the reaction products were dialyzed against large volumes of deionized water for three days at 4°C in the dark. The purified complex was then lyophilized. Fully substituted BSA-DNP which was obtained in the presence of high excess of 2,4-DNB-SA was first acidified (pH 2) to precipitate the dinitrophenylated protein which was then washed in cold KCl - HCl buffer (pH 1.7) until the washing solution became colorless. To free and remove unreacted 2,4-DNB-BSA encapsulated in the protein matrix the precipitate was dissolved in 1 M NaOH and moderately heated. Then the BSA-DNP fraction was again precipitated, washed, redissolved and passed through a Sephadex G50 column using phosphate buffer (pH 7.9). The fully derivatized BSA-DNP product was then extensively dialyzed against large volumes of deionized water and the salt-free protein was lyophilized. For reference underivatized BSA samples were subjected to identical treatment in 2,4-DNP-SA-free buffer solution and "purification" (desalting) procedures. Dinitrophenyl-conjugated MM anti-DNP IgE antibody was prepared from commercially available MM anti-DNP IgE antibody (Miles Scientific, Division of Miles Laboratories, Inc.) employing equilibrium dialysis technique. The dialysis experiments using 100 ul dialysis cells were performed against 10^{-6} M solution of the hapten (2,4-DNP, 2,6-DNP-Lysine, picric acid) at pH 8 in phosphate buffer, at 4°C and for 48 hours in the dark. The dialysis solution was at least 3 times changed and the system about 6-9 times gently shaken to avoid hapten depletion and protein denaturation during the 48-h incubation period. 1 mg/ml protein

concentration (in the dialysing buttons) was maintained during the entire dialysis process and thus, the spontaneous denaturization of the immunoprotein (due to low protein concentration) was probably prevented. However, denaturization could occur during washing and desalting when the immuno-protein concentration, ionic strength and pH, all were below those values which could be normally required to prevent denaturization. (It is believed that these preparative circumstances have the same effect on the conductivities of both conjugated and unconjugated MM anti-DNP IgE. Therefore, the difference expected in the respective conductivity measurements is not an artifact brought about by the difference in denaturization. However, the validity of this assumption will have to be scrutinized in future works.) Then samples (usually of 4-5) were collected and washed first with buffer solution and after that using deionized water several times. The residual salt contents of the desalted immunoprotein samples were calculated from the dilution factors and estimated to be one-to-two salt molecules per immunocomplex molecule. Control samples (unlabeled immunoprotein) were obtained by passing (simultaneously) MM anti-DNP antibody through identical experimental steps without adding hapten to the incubating buffer and final washing solutions.

Thin protein films were made from 1% (w/w) protein solutions by casting over an approximately 50 Å gap separating two silver electrodes on 1" x 1" x 1/16" quartz substrates (EBCO Products, Inc.). Only quartz carriers with less than 50 fA leak current at

70 V in vacuum qualified for further use. 1 ml of the stock solution was placed behind the leading edge of a vertically tilted slide glass equipped with two 41 μ thick Teflon spacers in 3 mm distance. The slide glass moved along the electrode gap at a constant velocity of 12 mm/min (Fig. 1). The films obtained were slowly desiccated in Petri-dishes at room temperature and then stored under vacuum at 10^{-5} torr. Scratch-free uniform films about 2 cm in length, 2 cm in width and 2 μ in thickness could be made in this way with quite good reproducibility. The casting was carried out using the apparatus shown in Fig. 1, in a plastic glove box to prevent dust deposition on the samples.

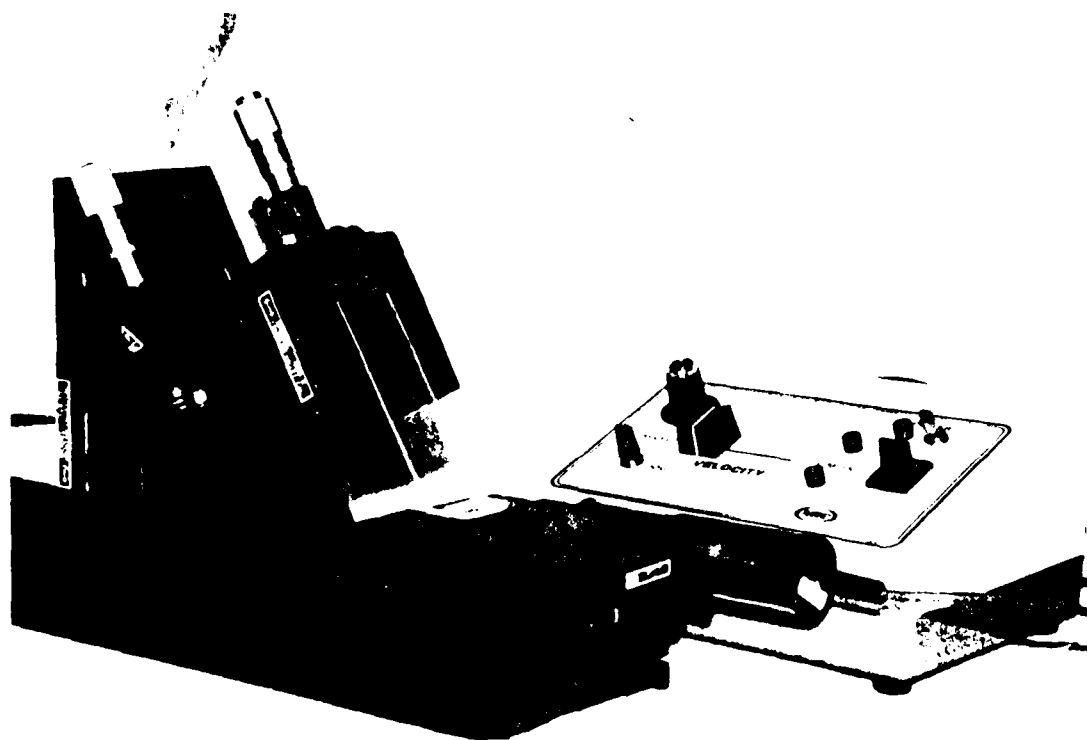


Figure 1. The full vision of the casting apparatus constructed from Newport precision mechanical elements.

Thin film samples were placed on a Teflon stage in evacuable plastic chambers (desiccators) in which the humidity could be controlled by concentrated solutions of recrystallized LiCl. Probably due to preferential vapor condensation in the narrow electrode gap, above 25% relative humidity the clean quartz substrates always displayed a very rapidly increasing conduction with increasing vapor pressure of water. Therefore, the measurements with protein films were restricted to 17.5, 20.0 and 23.0% relative humidities which were provided by LiCl solutions of 16, 15 and 14 M, respectively /6/. Exploratory conductivity experiments showed that 24 hour incubation was in all cases sufficient to reach steady state in the hydration - induced conductivity of the thin protein film samples. Small gold spirals (0.1 mm wire diameter) provided the electrical connections to the carefully shielded and isolated instrument cables. Sample chambers were kept in a grounded Faraday cage to avoid electric disturbances due to scattered external electromagnetic fields. The conductivity of dry samples was measured at 200 - 400 Pa pressure. Current was measured using Keithley Type 602 Electrometers.

Current measurements were carried out up to 70 V bias voltage at room temperature. To assure that a steady state in current was reached data were recorded in 10 minutes after imposing a given bias voltage. Due to the geometric conditions exact resistance values could not be calculated. If not otherwise noted current values obtained at 70 V will be considered as the measure of conductivity and used for comparative purposes. The overall

reproducibility of measurements was limited by the film casting technique and can be conservatively estimated as 30%.

RESULTS

BSA-DNP complexes with known stoichiometry provided the scaling and calibration for the thin film measurements with MM anti-DNP IgE antibody complexes. Therefore, first the results obtained with BSA-DNP complexes will be described.

Reference Data

The absorption spectra of the purified BSA-DNP complexes displayed two prominent absorption bands, the one centered between 270 - 280 nm and the other at ~ 360 nm, with a small shoulder above 400 nm. The latter is very similar to that reported for -DNP-lysine and dinitrophenylated bovine gamma globulin. The significantly lower shoulder may be due to the different participation of nitro groups in secondary interactions (very likely hydrogen bonding). The lack of any feature at 255 nm (typical of 2,4-DNB-SA) and of the strong shoulder above 300 nm (characteristic of 2,4-DNP in polar environment) indicate that neither free 2,4-DNB-SA nor free 2,4-DNP was present in spectrophotometrically detectable amounts in the purified BSA-DNP products. There was only a minor spectroscopically observable heterogeneity in the microenvironments of, and practically no interaction between, the chromophores even in the fully derivatized complex which contains at least 80 2,4-dinitrophenyl groups substituted in primary amine moieties. This suggests spectral additivity and permits the spectroscopical verification

of the extent of average substitution in complexes with different stoichiometry. The 360 nm band exhibits a monotonic progression with increasing substitutions as it is shown by the spectra normalized at 275 nm in Fig. 2. Provided that spectral additivity holds the 360 nm peak heights of the normalized spectra relate to the average number of substitution per BSA molecule, \bar{n} , according to the following expression:

$$\left\{ \frac{\epsilon_{360}^{all}}{\epsilon_{275}^{all}} \right\}^{-1} = \frac{1}{\bar{n}} \frac{\epsilon_{275}^{BSA}}{\epsilon_{360}^{BSA-DNP}} - \frac{\epsilon_{275}^{BSA-DNP}}{\epsilon_{360}^{BSA-DNP}}$$

where ϵ_{λ}^x is the extinction coefficient of species x at wavelength λ . The experimental verification of this relationship is given in a linearized form in Fig. 3. The intercept is close to the value of the corresponding ratio obtained from direct measurements (0.75). All these provide a favorable support for the BSA-DNP stoichiometries assumed on the basis of the reaction conditions.

Dry protein films, (both BSA and MM anti-DNP IgE antibody, no matter whether or not they had been derivatized and/or conjugated), always exhibited an extremely low conductivity permitting sample currents close to or well below the leakage (typically 10 fA). Therefore, to directly determine the conductivity of dry samples was impossible. When the samples were

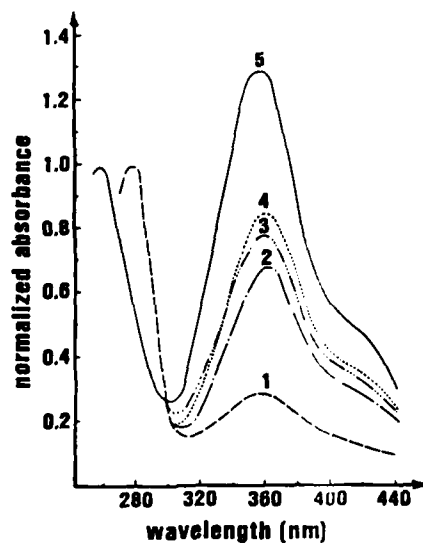


Figure 2. Near UV absorption spectra dinitrophenylated bovine serum albumin samples derivatized at different BSA: 2, 4-DNP stoichiometric ratios: 1-1:1; 2-1:2; 3-1:4; 4-1:8 and 5-1: 80 (fully derivatized). The spectra were taken in water, at p and, normalized at the 275 nm maximum.

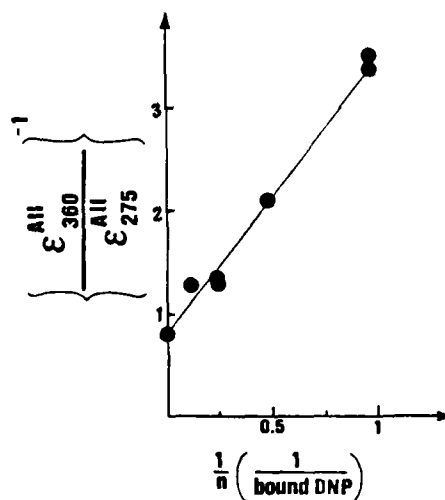


Figure 3. Spectrophotometric verification of the stoichiometric compositions of dinitrophenylated bovine serum albumin samples.

equilibrated with low humidity atmosphere the conductivity increased to well measurable levels. The higher the ambient humidity was the more pronounced increase in the conductivity was observed. This is in a complete qualitative agreement with previous findings on powder samples /7-11/. No attempt was made to quantitatively compare these two system regarding their response of their conductivity to moisture adsorption in the present studies. At low relative humidities (below 25%), when any interference of the quartz substrate was certainly absent, the current - voltage characteristics were found linear for all hydrated BSA and BSA-DNP films (Fig. 4). Moreover, it was very

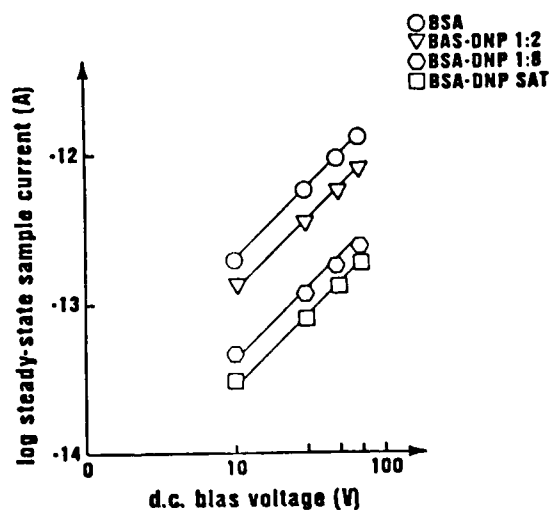


Figure 4. Current - Voltage characteristics of ultrathin films of bovine serum albumin and dinitrophenylated bovine serum albumin derivatized at stoichiometries 1:2, 1:8 and 1: 80 (fully derivatized), respectively, equilibrated with and measured in, ambient atmosphere of 17.5% relative humidity.

consistently observed that BSA-DNP films possessed definitely

lower conductivity than the reference BSA samples, at the same hydration, as it is also seen in Fig. 4. The more extensive the substitution the greater (but not proportional) the change in conductivity. Water clearly behaves like a developer in making hidden (unmeasurable) changes in dry-state conductivity observable and measurable. An interesting pattern becomes visible if one plots the ratio of the conductivities of BSA and BSA-DNP samples as a function of humidity for complexes with different BSA: 2,4-DNP stoichiometry. Such a representation of data is given in Fig. 5 in a three-dimensional plot for complexes with

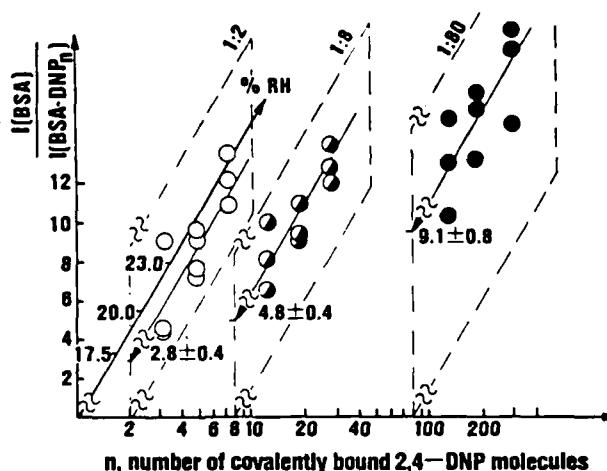


Figure 5. Dependence of the conductivity ratio $I(\text{BSA})/I(\text{BSA-DNP}) = \sigma(\text{BSA})/\sigma(\text{BSA-DNP})$ upon the relative humidity of the ambient atmosphere (RH) and, stoichiometric composition (n) of the complexes.

stoichiometries 1:2, 1:8, and 1:~80 (fully derivatized), respectively. It is strikingly apparent that the conductivity ratios are grouped in such a way that they form three lines parallel to the humidity axis, i.e. they seem to be independent of

humidity in each case. The numerical values for the average of the respective ratios are 2.8 ± 0.4 for the 1:2 complex, 4.8 ± 0.4 for the 1:8 complex and 9.1 ± 0.8 for the fully derivatized sample.

MM Anti-DNP IgE Antibody Studies

Due to unforeseen difficulties in obtaining reliable and regular supply of antibody as well as the lack of expected cooperation of the supplier [12] we had more limited opportunities to perform systematic conductivity experiments with MM anti-DNP IgE antibodies. The fundamental problems stem from the early observations that conductivity data obtained with this monoclonal immunoprotein (unconjugated and conjugated) displayed a much wider scatter than those for BSA-based samples. Especially, large and apparently unacceptable deviations were observed for immunoprotein samples purchased and delivered at different times. Furthermore, systematic deviations were found in the case of samples from the same batch when they were delivered at different times. These latter observations are true for the majority of experiments performed prior to December 15, 1985. Then it turned out that the immunoprotein samples were stored and shipped by the company (Miles Laboratories) under improper conditions. This happened although we repeatedly asked them to make sure that the samples would be kept frozen and on dry ice from sealing the vials at the production site until arrival in our laboratory. Furthermore, it seems also plausible that the significantly larger molecular weight (190,000) and the more sensitive native structure

of the antibody could also contribute to the larger scatter in conductivity data. Due to these circumstances the conductivity measurements on MM anti-DNP IgE antibody films could not be evaluated in such a relatively simple and straightforward manner as in the previously described BSA work. It was concluded that for the immunoprotein the evaluation of measurements data would be more appropriate using Poisson distribution rather than the traditional normal distribution. Similarly to the BSA case the conductivity (current) ratios $\sigma(\text{unconj.})/\sigma(\text{conj.})$ were formed at each humidity and they were subjected to statistical evaluation based upon Poisson statistics. If so, the probability for observing a conductivity ratio $\sigma(x=nx_0)$ which is defined as

$$f(x) = f(nx_0) = \frac{\lambda^{(nx_0)} e^{-\lambda}}{(nx_0)!} = \frac{\lambda^x e^{-\lambda}}{x!}$$

where λ is a parameter equal to the mean of $x(=nx_0)$ and x_0 an arbitrary chosen unit. If $f(x)$ the relative frequency of $\sigma(x)$, which is determined from experiments then the representation

$$\ln \{ f(x) \cdot x! \} = x \ln \lambda - \lambda$$

of measurements data should result in a straight line in a $\ln \{ f(x) \cdot x! \}$ vs. (nx_0) plot, which has a slope of $\ln \lambda$ and an intercept with the vertical axis at $-\lambda$. Thus λ can be determined (either from the slope or the intercept) and is considered as the mean value of the conductivity ratios. Any significant deviation of the mean values from unity indicates that

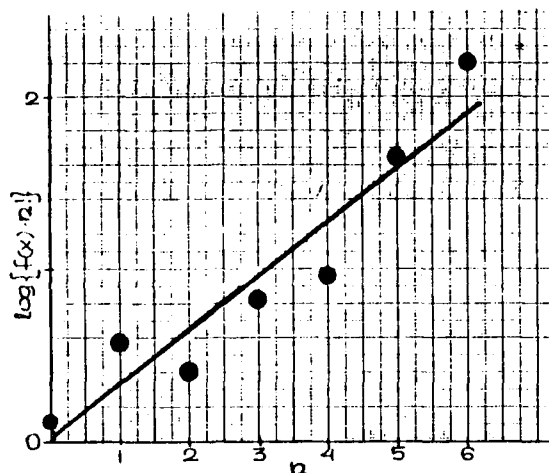


Figure 6. Poisson evaluation of experimental conductivity (current) ratios for monoclonal mouse anti-DNP IgE antibody - TNP (picric acid) conjugate at 20% relative humidity.

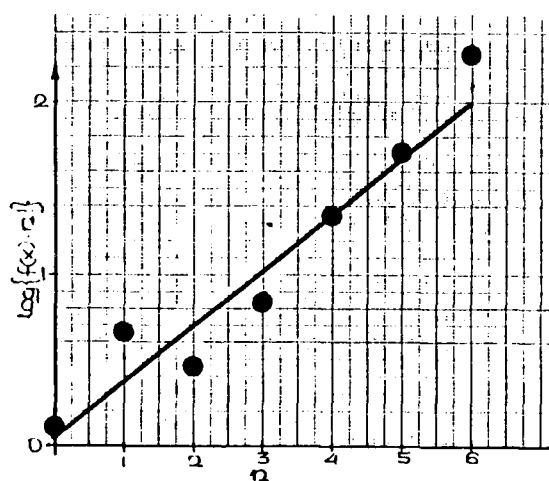


Figure 7. Poisson evaluation of experimental conductivity (current) ratios for monoclonal mouse anti-DNP IgE antibody - TNP (picric acid) conjugate at 23% relative humidity.

Table 1

Conductivity (Current) ratios $\bar{\sigma}(\text{conj})/\bar{\sigma}(\text{unconj})$ for Monoclonal Mouse anti-DNP IgE Antibody complexed with 2,4-DNP-Lysine and/or TNP (picric acid) ligands. The regression coefficients are given in parentheses.

Ligand	Humidity		
	17.5%	20%	23%
2,4-DNP-Lys	1.5	1.9	1.9
	(0.99)	(0.89)	(0.96)
Picric Acid	--	2.1	2.2
		(0.96)	(0.92)

In the case of 2,4-DNP no specific binding was detected in conductivity measurements.

the binding of hapten changes the conductivity of the immunoprotein. Such a presentation of two sets of conductivity ratios for MM anti-DNP IgE antibody conjugated with TNF hapten at 20 and 23% relative humidities is given in Figs. 6 and 7. It is clear from these lots that the experimental data are well arranged along straight lines, as accepted on the basis of the preceeding considerations. This behavior was typical of most sets of conductivity ratios obtained from independent experiments at all humidities applied. The straight lines were fitted by parabolic least square method (Gauss-Jordan) and the slopes and intercepts were computed. The values obtained from the slopes and the intercept, respectively, were identical within experimental error indicating the appropriateness of the procedure. The results of such evaluations are summarized in Table 1. These figures strongly suggest that the binding of various 2,4-DNP-based haptens to MM anti-DNP IgE antibody brings about a change in conductivity similar in magnitude to the change observed with derivatized BSA model systems.

DISCUSSION

Despite the extensive experimental and theoretical efforts the nature and the propagation mechanisms of mobile charge carriers in proteins are still obscure in both solid and their native states. Dry crystalline proteins have been found to possess conformations not appreciably different from those in aqueous solutions /13-14/. Several enzymes not only preserve their native conformations and biological activities but are also

capable of exercising their highly specific catalytic functions in crystalline state /16-19/. These findings certainly justify the solid state physical approach to gain insight into the fundamental properties of charge carrier transport in proteins. However, it is not straight-forward to form an opinion about the possible relevance of any of such findings to biological functions, as yet.

Preliminary studies on powder samples /20/ suggested that the substitution may result in marked alterations in the conduction properties. Detailed experiments /1/ showed that charge transport processes at the electrode - sample interfaces can exert significant influence on the data obtained in d.c. conductivity measurements on powder samples. Although the origins, the nature and the mechanism of these interfacial processes are not known as yet, it is plausible to assume that similar (if not basically identical) events occur at metal - protein thin film interfaces. The extents and the possible involvement of interfacial processes in the derivatization-induced change in conductivity is not known either. Therefore, d.c. measurements were performed on thin solid films of BSA and BSA-DNP, in which interfacial phenomena, if there are any, would certainly dominate the results. These films in slightly hydrated state exhibit a remarkable reproducibility in their d.c. conductivity properties with linear current - voltage characteristics up to at least 70 V bias potential (about 10 kV/cm effective field in the electrode gap). The linearity (Fig. 4) suggests that high-field effects were certainly absent under the present conditions.

Similarly to the results of the pilot studies it was found that a rather moderate chemical modification of BSA, substitution of a 2,4-dinitrophenylate group into one or two primary amines brings about marked changes in the conductivity of thin protein films. This change, a decrease, in conductivity is normally unobservable in/on dry films, the sample resistance being extremely high. However, this latent change can be developed by equilibrating the samples with, and performing the measurements in, ambient atmosphere with less than 25% relative humidity. Such hydration experiments reveal that gradually proceeding derivatization of BSA results in an apparently gradual decrease in the overall conductivity, which is of saturation-type rather than linear. The film conductivity increases monotonically with rising moisture content as it was expected on the basis of previous experiments on protein powder [1,7,8,11]. Provided that, as in powder samples, the conductivity of hydrated protein films, σ_H , also obeys the Spivey relationship [22] and that, the amount of water adsorbed into the film is proportional to the ambient humidity in the range of relative humidity applied, then

$$\sigma_H = \sigma_D \exp(\alpha w),$$

where σ_D is the dry state conductivity, α a constant and w the relative humidity. Then the conductivity ratios plotted in Fig. 4 will be independent of hydration only if the respective exponential terms (and thus, the exponents) are identical and in this case

$$\frac{\sigma_H(\text{BSA})}{\sigma_H(\text{BSA-DNP})} = \frac{\sigma_D(\text{BSA})}{\sigma_D(\text{BSA-DNP})}$$

for all stoichiometries. Thus, the averages determined from, and indicated in, Fig. 4 measure the extent of the substitution - induced decrease of protein conductivity for the given stoichiometric complex (in the dry and slightly hydrated states).

The most important practical outcome of these studies is obviously the fact that the covalent binding of two small molecules to, i.e. the local modification of the protein at two primary amine groups in, BSA molecule brings about a well measurable change in the d.c. conductivity of protein thin films.

Several types of charge carrier transport processes have been proposed to operate in proteins hydrated to different extents. These include a great variety of semiconduction mechanisms and proton transfer processes involving both polypeptide backbone and side groups /21/. Furthermore, recent experimental and theoretical studies /11/ provided a great deal of support in favor of a hopping rather than coherent wave packet type motion of charge carriers in proteins. Whatever the nature and propagation mechanism of charge carriers in the dry and slightly hydrated BSA and BSA-DNP complexes are, the conduction phenomena observed and reported in this communication are very likely dominated by the interfacial charge transfer step between the Ag electrode and protein film /1/. Therefore, they may not reflect any peculiarity of the bulk conduction but rather those of the charge carrier injection process. The extremely high sensitivity (two small molecule per one BSA molecule), the stepwise response and the apparently saturation-like characteristics of the

derivatization-induced drop in d.c. conductivity strongly suggest that /22/:

- 1) Only a finite number of sites may exist on the surface of protein molecules through which charge carriers can be preferentially injected (ejected) from (into) the electrode into (from) the protein. Similar considerations may be true for the interprotein charge transfer as well.
- 2) The number of these charge carrier transfer sites must be relatively small, the (partial) blocking of one or two of them per protein being detectable.
- 3) The preferential charge carrier transfer sites may not be completely equivalent to each other due to either possible minor differences in their actual composition and structure, or the differences in their positions with respect to the injecting electrode and/or to neighboring proteins and their corresponding charge carrier transfer sites.
- 4) Primary amine groups are probably essential components in the formation of the preferential charge carrier transfer sites on proteins.

The change in conductivity of MM-anti-DNP IgE antibody due to hapten binding appears to be very reminiscent of that produced by the derivatization of BSA. The extent of the change is close to that observed at 2:1 stoichiometry, but the reproducibility of data seems to be poorer. Besides the mentioned uncertainties in sample quality the nature of the immunoprotein can be also

accounted for the larger scatter. Let us pretend that the charge injection into (and transport in) MM anti-DNP IgE is similar to that in BSA, which was discussed above. Since the immuno-protein is about 3 times larger (heavier) than BSA it can be imagined that the binding sites may be effectively masked or even deeply burried in the protein and therefore they participate in the charge injection and transfer process less probably than in BSA. This leads to a less visible alteration of conductivity on the one hand and a naturally wider scatter in measurements data on the other. Moreover, the adsorption properties of the globular BSA and the delicate MM anti-DNP IgE on the substrate can be significantly different /23/ and the absorption-induced changes may affect the conductivity measurements to different extents. Further investigations into this problem are needed to understand the role and consequences of adsorption induced structural changes on conductivity results. Irrespective of this unknown factor it is believed that hapten binding itself do change the conductivity of MM anti-DNP IgE thin films. This means that monoclonal antibodies could form, in principles at least, the selective sensory element (receptor) in chemical microsensors. Besides the technical difficulties which are rather serious, further problems which are apparently of academic interest should be solved in order to utilize the potentials of such systems.

IV. CONCLUSIONS & SUGGESTIONS

Our investigations performed during the grant period between October 1st, 1982 and February 28th, 1986 have basically demonstrated that the utility of d.c. conductivity measurements on thin immunoprotein films as a probe of antigen/hapten binding by monoclonal mouse anti-DNP IgE antibody. Upon binding to the antibody, appropriate nitrophenyl ligands (2,4-DNP-Lysine, TNP) bring about typical changes in the d.c. conductivity: it decreases by about a factor of two. Model compounds (BSA and its derivatized forms) were used to provide information about the possible mechanism of resistance lowering by DNP-based haptens. Convincing support if not evidence was obtained for a discrete site assisted rather than a band-type (semiconductor like) charge carrier transport mechanism in proteins films. It is very likely that primary amine groups are involved in forming preferential charge transfer sites in proteins. The similarities in the binding (derivatization and/or conjugation)-induced changes in conductivity indicate that interactions between primary amine groups (at the recognition site) and DNP is a dominant feature in this immune complex system. The relatively large scatter in measurements data, besides the inherent sensibility of the protein structure, can be related to the partial masking of the binding site by the bulky protein. Further studies into this question are certainly desired and suggested. Experiments with separated and recombined heavy and light chains in conjugated and unconjugated

forms as well as on partially digested immunoprotein are suggested to perform in order to clarify the possible role of the bulk of the protein in the conduction process and in its response to hapten binding. Investigations on other hapten - monoclonal antibody systems are also suggested to reveal further aspects of hapten (antigen)-induced conductivity changes in immunoproteins. A deeper understanding of charge transport mechanism in proteins, in general, is also desirable especially if practical applications are planned.

Finally we would like to call the attention to the unreliability of commercial monoclonal immunoprotein sources. Therefore, in any further work sufficient financial, technical and scientific capacity should be planned to produce monoclonal antibodies in desired purity, quality and quantity.

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